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IMMUNOLOGICAL APPROACH TO THE IDENTIFICATION AND DEVELOPMENT OF VACCINES TO VARIOUS TOXINS

ANNUAL REPORT

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Monoclonal antibodies specific for saxitoxin and tetrodotoxin with higher binding affinities than those generated under this contract are still being sought for. Attempts to generate internal image monoclonal anti-idiotypic antibodies specific for the highest binding anti-saxitoxin and anti-tetrodotoxin antibodies are continuing. A monoclonal anti-idiotypic antibody, designated DE8, specific for a protective anti-mycotoxin T-2 monoclonal antibody (HD11) was generated. Administration of DE8 into syngeneic BALB/c mice induced a protective antibody response against the in-vitro and in-vivo toxicity of T-2.

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FOREWORD

In the conducting the research described in the report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources - National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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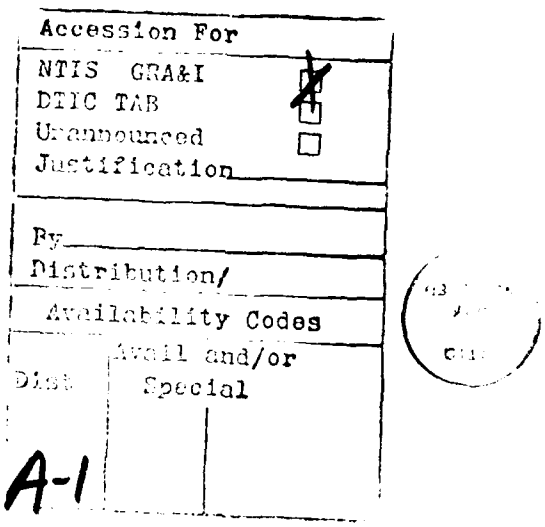

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I. INTRODUCTION

The sodium channel blockers, saxitoxin (STX) and tetrodotoxin (TDT), and the protein synthesis inhibitor mycotoxin T-2 are potent, nonproteinaceous small molecular weight biological toxins. Because of their small size, they do not generally induce an antibody response with a high average binding affinity, even when conjugated to large protein carriers, which could protect against the toxicity induced by these biological toxins. Moreover, the current methodology for conjugation of toxins to protein carriers usually result in the formation of unstable complexes with the potential release of the native toxins in their toxic form. Therefore, it is impractical if not impossible to develop effective vaccines against these biological toxins using conventional technology such as synthetic peptides or recombinant technology. The very toxic nature of these biological compounds also preclude their use as immunogens in the induction of effective protection. Because of these difficulties, we have attempted to develop anti-idiotype (anti-Id) based vaccines for the induction of protective immunity against STX, TDT and T-2. Our approach involves the generation of high affinity monoclonal antibodies (mAbs) specific for the respective toxins which are protective against toxicity. These anti-toxin antibodies (Abl) are then used to generate syngeneic monoclonal anti-Id antibodies which serologically mimic the nominal antigen (or toxins). The generated anti-Id antibodies are then assessed for their ability to mimic the antigen and to induce in mice a protective immunity against toxicity.

Two specific anti-STX mAbs were generated that possess affinity constants (K_a values) of approximately 10^6 M^{-1}. They are designated S1A5 and S3E.2, and are of the IgM_k and IgG_{1k} isotypes, respectively. Two stable and specific clones were also generated against TDT among several thousand hybrids tested. The mAbs, termed TD13a1 and TD2C5, are IgG_{1k} mAbs with K_a's values approaching 10^7 M^{-1}. In spite of their relatively low K_a's values, these anti-STX and anti-TDT mAbs were found to protect against the toxicity induced by STX and TDT, respectively.

In the T-2 mycotoxin system, an IgG_{1k} anti-T-2 mAb (HD11) was isolated that had a relative affinity binding constant of approximately 4.8×10^8 M^{-1}. This mAb was shown to protect against the <u>in-vitro</u> toxicity of T-2 mycotoxin. HD11 mAb was used to generate a monoclonal anti-Id antibody, designated DE8, which when injected into mice induced a significant protective immunity against the <u>in-vitro</u> and <u>in-vivo</u> toxicity induced by T-2.

II. RESULTS

A. Studies with Saxitoxin.

The results of studies involving the sodium channel blocker STX with respect to generation of specific mAbs and their ability to inhibit STX toxicity in-vitro were discussed in previous annual reports and published (1). In summary, STX was conjugated to keyhole limpet hemocyanin (KLH) and used to immunize BALB/c mice. Anti-STX antibodies were detected in serum by an enzyme-linked immunosorbent assay (ELISA) within a week or two after the first immunization (1). Spleens from immunized mice were fused with NS-1 myeloma cells and approximately 7000 resultant hybrids were screened by ELISA for reactivity to STX. Two stable hybrids were isolated, subcloned, and characterized. These hybrids, termed S1A5 and S3E.2, secreted specific anti-STX antibodies that did not recognize the closely related toxin TDT, as determined by competition ELISA. The S1A5 mAb was of the IgM_k class and S3E.2 of the IgG_{1k} subclass with affinity constants of approximately 10^6 M^{-1}. The protective ability of these antibodies was tested by a competitive displacement assay for [^{3}H]STX binding on rat brain membranes. Purified S3E.2 strongly displaced [^{3}H]STX binding, whereas S1A5 weakly inhibited [^{3}H]STX binding to membranes. One nanomole of S2E.2 or S1A5 was able to bind 0.03 nmol or 0.005 nmol, respectively, of STX. The S2E.2 mAb offered partial protection against STX-induced reduction of peripheral nerve action potential in rat tibial nerve when administered in situ at concentrations 10-to 30-fold greater than STX. The S1A5 mAb, despite its ability to inhibit STX binding in vitro, was completely ineffectual in situ (1). These antibodies, particularly S3E.2, thus represent potentially useful reagents for neurobiologic research, detection of toxin contamination, and diagnosis of poisoning, and may provide protection against the toxicity of STX in vivo.

Because of the relatively low affinity constants of the generated anti-STX mAbs, two approaches were taken in an attempt to increase the affinity of binding (see second annual report). Briefly, the first approach involves the in-vitro secondary immunization of in-vivo STX-primed mouse spleen cells prior to fusion. Mice were also immunized with STX-KLH coupled via the linker N-succinimidyl 3-2 (pyridyldithio)proprionate (SPDP). However, neither approach resulted in higher affinity mAbs than the ones described above. The S3E.2 anti-STX mAb has been used to immunize mice for the generation of internal image monoclonal anti-Id antibodies.

B. Studies with Tetrodotoxin.

Monoclonal anti-TDT antibodies were generated as described above for anti-STX mAbs (2). Spleens from immunized mice were fused with NS-1 mouse myeloma cells and ~ 9,329 resultant hybrids were screened by ELISA for reactivity to TDT. Two stable hybrids, termed TD13a1 and TD2C5, secreted specific anti-TDT antibodies that recognized TDT but not the related sodium channel blocker, saxitoxin (STX), as determined by competition ELISA. Both antibodies were of the IgG_{1k} subclass with K_a's approaching 10^7 M^{-1}. The inhibitory ability of these antibodies was tested by a competitive displacement assay for [^{3}H]STX on rat brain membranes. Both antibodies strongly inhibited TDT binding to membranes. A nanomole of TD2C5 was able to

- 1.8 nmol of TDT, whereas a comparable amount of TD13a1 bound half as much. Furthermore, TD2C5 was able to protect against TDT-induced reduction of peripheral nerve action potentials in rat tibial nerve when administered in situ. Because of the protective efficacy of TD2C5 anti-TDT mAb in-vitro and in-situ, TD2C5 mAb was conjugated to KLH and used to immunize BALB/C mice and rabbits for the production of anti-Id antibodies (see second annual report). A number of fusions was performed with spleen cells from mice immunized with TD2C5-KLH. To date, no monoclonal anti-Id antibody with internal image has been isolated. Efforts are continuing to generate anti-Id with vaccine potential.

C. Studies with T-2 Mycotoxin.

Using T-2 conjugated to ovalbumin (OVA), generously provided by Dr. J. Hewetson (USAMRIID, Fort Detrick, Frederick, MD), to immunize BALB/C mice, a monoclonal anti-T-2 antibody (HD11) was generated that had a relatively high binding affinity constant. HD11 anti-T2 mAb was also found to be protective against the T-2-induced cytotoxicity on the human epidermoid carcinoma cell lines Hep-2 and KB (3). Rabbit anti-Id antibodies specific for HD11 were generated and characterized (see second annual report and 3). HD11 conjugated to KLH was also used to immunize syngeneic BALB/C mice for the generation of monoclonal anti-Id antibodies. One mAb specific for HD11 anti-T2 was isolated. The characteristics of this anti-Id mAb, designated DE8, have been described in details elsewhere (4 and second annual report). Briefly, DE8 anti-Id mAb is specific for HD11 anti-T-2. DE8 recognizes an Id determinant on HD11 associated with the T-2 binding site since the interactions between DE8/HD11 are inhibited by free T-2 mycotoxin. Addition of DE8 mAb to HD11 completely abrogated the protective effect of HD11 against T-2 cytotoxicity, suggesting that the binding of DE8 to HD11 prevented the latter from binding to and inactivating T-2 (4). Administration of DE8-KLH or unconjugated DE8 into BALB/C mice resulted in the induction of a specific and significant circulating anti-T-2 antibody response characterized by HD11 Id-positive antibodies. Moreover, the DE8-induced anti-T-2 antibody response was able to abolish T-2 cytotoxicity as determined by [^{3}H]-leucine uptake of the Hep-2 and KB cells (4). Taken together, these results suggest that DE8 anti-Id mAb possesses the "internal image" of the nominal antigen (T-2) and that it can serologically mimic T-2 in the induction of an anti-T-2 immune response.

Encouraged by these results of the in-vitro protection, we assessed the ability of DE8-KLH to induce an anti-T-2 antibody response that would protect mice against a lethal challenge with T-2. Groups of five BALB/c mice each were immunized with four injections of DE8-KLH or control anti-Id mAb. One week after the last immunization, the mice were challenged intradermally with various doses of T-2 mycotoxin. The results are presented in Table 1. In our hands, the LD_{50} of T-2 in mice challenged intradermally is approximately 5.0 mg/kg body weight Mice actively immunized with DE8-KLH were protected against T-2 succumbed to T-2 toxicity, all the remaining DE8-KLH-treated mice, even the groups that received 20 mg/kg (4 times the LD_{50}), survived. The LD_{50} of the control antibody group was comparable to that of the untreated control group. Experiments using higher doses of T-2 will be performed to determine the LD_{50} of DE8-KLH-immunized mice. This represents to our knowledge the first demonstration of anti-Id-induced protective immunity against a small molecular weight biological toxin.

III. Summary

Our demonstration of the successful use of anti-Id based vaccine in inducing a protective immunity against T-2 mycotoxin provides further evidence for the ability of anti-Id antibodies to serologically mimic nominal antigens of small molecular weight and of a nonproteinaceous nature. It is hoped that our efforts in the STX and TDT systems will also yield positive results.

Table 1

Administration of DE8 Anti-Idiotype Protects Mice Against T-2 Toxicity In-Vivo

Mice immunized with	T-2 Mycotoxin (ug/ml)	Survival ratio[a]
Control Anti-Id	5.0	3/5
	10.0	4/5
	15.0	5/5
	20.0	5/5
DE8 Anti-Id	5.0	0/4
	10.0	1/5
	15.0	0/5
	20.0	0/5

[a]Number of dead mice/total number of mice tested. T-2 mycotoxin was administered intradermally.

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